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Antimicrobial, antioxidant and alginate potentials of *Dictyopteris polypodioides* (Dictyotales, Phaeophyceae) from the Moroccan Atlantic coast

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ABSTRACT

The aim of this work was to evaluate antibacterial, antifungal and antioxidant activities as well as alginate yield and quality of the brown seaweed Dictyopteris polypodioides, collected from the Moroccan Atlantic coast. The results showed that Dictyopteris polypodioides exhibited high activity against the most human pathogenic bacteria tested (Staphylococcus aureus, Bacillus cereus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Enterococcus faecalis, Salmonella sp.). The species demonstrated also an important antifungal activity against both species of the pathogenic fungi Candida and the most tested phytopathogenic fungi (Verticillium dahlia, Fusarium oxysporum, Fusarium graminearum, Botrytis cinerea and Geotrichum sp.). The high total phenolic content (27.54±0.59 % dw) has been correlated with the important DPPH scavenging activity (84.13%) and the low EC_{50} *(0.43mg/ml). Furthermore, sodium alginate yield was about 21.92 % dw considered within the range of those reported from other worldwide alginophytes used in the industry. Infrared spectroscopy analysis shows that the obtained spectrum exhibits strong similarities to the commercial sodium alginate, especially at the anomeric region of fingerprint (950-750* cm^{-1}*).* ^{1}H *NMR spectroscopy demonstrated an alginate polymer dominated by guluronic acid (M/G = 0.7) and alternating blocks MG and GM (*$F_{MG} = F_{GM} = 0.41$*). In conclusion, Dictyopteris polypodioides seems to be a promising source of bioactive compounds and alginates in Morocco with controlled and ecologically sustainable harvesting.*

Keywords: *Dictyopteris polypodioides*, antibacterial Activity, antifungal Activity, antioxidant Activity, alginates.

INTRODUCTION

Marine macroalgae, commonly known as seaweeds, are conspicuous and dominant features in marine ecosystems [1]. Fresh or dried forms of seaweeds have been used by East Asian countries like Japan, Korea, China, Vietnam, Indonesia, and Taiwan for their daily cuisines as a condiment or a vegetable in soups, stews, or with rice or noodles [2]. Owing to the unique physicochemical properties of seaweed-derived compounds, there are enormous potential applications the fields of food, feed, biomedical, agricultural, environmental [3,4,5] and other industrial applications. Seaweeds are also an important source of phycocolloids such as agar, carrageenan, and alginate. These compounds are used as gelling, stabilizing, and thickening agents in the pharmaceutical, food, textile, paint, and varnish industries [6]. Otherwise, numerous structurally unusual secondary metabolites, such as sesquiterpenes [7, 8] diterpenes [9, 10] meroterpenoids [11], C15-acetogenins [12], phlorotannins [13] and steroids [14,15], have been frequently reported from various species of seaweeds. It is noteworthy that many of the compounds have demonstrated various biological activities, widely ranging from antitumor [16], antibacterial [17, 18, 19],

antioxidant [20], anti-inflammatory properties [21], anticoagulant and antiviral activities [22]. In particular more than 1140 secondary metabolites have been reported from Phaeophyceae, the characteristic compounds of these brown algae include diterpenes, phlorotannins, and small C11 acetogenins, all with very little halogenations [23]. For example, *Dictyota menstrualis* which belongs to the Dictyotaceae Family is characterized by a variety of chemical constituents, including diterpenes showed antiviral activity [24].

From the same Family, *Dictyopteris polypodioides* (De Candolle) J.V. Lamouroux 1809 (Dictyotales, Phaeophyceae) is a brown seaweed with a worldwide distribution. In Morocco, the species is well distributed and abundant in spring to mi-summer all around the Mediterranean shorelines and from the North to the central Atlantic Moroccan coasts (Figure1). The species shows generally yellow-brownish leaf-like dichotomously forked thalli reached up to 30 cm in shallow large intertidal pools. Despite its worldwide distribution, the potential use of *Dictyopteris polypodioides* in pharmacology or as an alginate target remains not explored due to a lack of research data. Recently however, one of the few reports about bioactivity was performed by Karaki et al. [25] on antioxidant and anticoagulant activities of some polysaccharides isolated from this species. Otherwise, recent studies on other species belonging to *Dictyopteris* genus have investigate antitumor, antioxidant and antimicrobial activities [26,27]. In this context, *D. polypodioides* was prospected for its antioxidant and biological activities of methanolic extracts against bacteria, yeasts and fungi with clinical and agricultural relevance as well as for alginate yield and chemical properties.

MATERIALS AND METHODS

2.1. Sampling and extract preparation

The seaweed *Dictyopteris polypodioides* was collected on the Moroccan Atlantic coast at El Jadida shoreline (Figure 1). Samples were brought to the laboratory and washed with distilled water to remove sediment and epiphytic organisms. Afterwards, algal biomass was dried in an oven at 60°C for alginate extraction or in freeze dryer for biological activities. The lyophilized sample was submitted to extraction with 80% methanol at 1:10 (m/v). The methanolic extract of *Dictyopteris polypodioides* was concentrated by evaporation and used to quantify total phenolic content (TPC) and to evaluate antimicrobial and antioxidant activities.

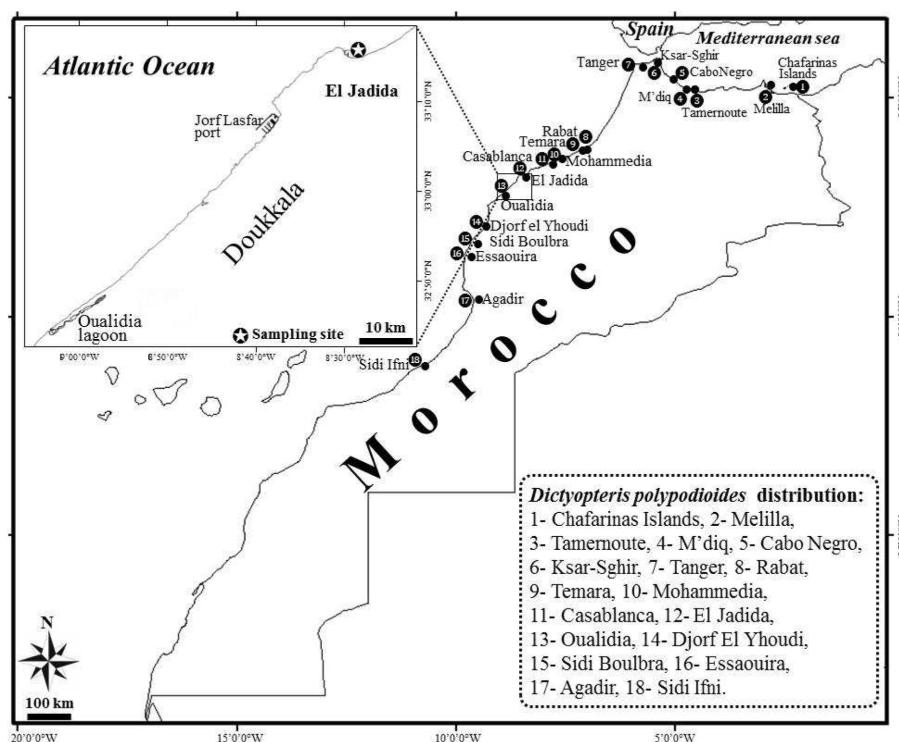

Figure 1: Geographic distribution of *Dictyopteris polypodioides* populations in the Atlantic and Mediterranean shorelines of Morocco. Top left inset: location of the sampling site (✪)

2.2. Determination of Total Phenolic Content

Total phenolic content was determined using Folin-Ciocalteu reagent as described by Heffernan et al., [28] using phloroglucinol as a standard. An aliquot of the methanolic extract (100 µl) was mixed with 50 µl of Folin-Ciocalteu reagent. To this was added 200µl of sodium bicarbonate solution (20%). The mixture was incubated at temperature

room and kept in the dark for 30 min. The absorbance of the mixture was measured at 735 nm using a spectrophotometer UV-Visible Metashe 5200 HPC. The Total phenolic contents was calculated and then expressed as phloroglucinol equivalent in % dry sample. All experiments were performed in triplicate.

2.3. DPPH radical scavenging assay

The antioxidant activity of *Dictyopteris polypodioides* methanolic extract was established as diphenylpicrylhydrazyl (DPPH) free-radical scavenging according to the method of Blois [29] with slight modification. DPPH free radicals have a purple color which turns into yellow when reduced by antioxidants. An amount of DPPH (0.06 mM) was dissolved in methanol and added to seaweed extract at different concentrations (5 to 100 mg/ml). The samples were incubated in the dark at room temperature for 30 min. After this time the absorbance was measured at 517 nm using a spectrophotometer UV-Visible Metashe 5200 HPC. The results were compared against a negative control (0.06 mM DPPH solution only) and positive controls (BHT and ascorbic acid). The percentage of DPPH radical scavenging was calculated with the following equation:

$$\text{DPPH scavenging activity (\%)} = [(Ac-As)/Ac] \times 100$$

where Ac is the absorbance of the negative control (methanol with DPPH solution) and As is the absorbance of the sample.

2.4. Antimicrobial activities

2.4.1. Microorganisms tested

The antibacterial activity of *Dictyopteris polypodioides* methanolic extract was tested against seven pathogenic bacteria including Gram positive species: *Staphylococcus aureus*, *Bacillus cereus*, *Enterococcus faecali,* and Gram-negative species: *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella* sp, and *Klebsiella pneumoniae*. The antifungal activity was studied using four yeast species: *Candida albicans*, *Candida glabrata*, *Candida krusei* and *Candida parapsilosis* and five phytopathogenic fungi: *Verticillium dahlia, Fusarium oxysporum, Fusarium graminearum, Botrytis cinerea and Geotrichum* sp.

2.4.2. Agar disc diffusion assay

The antimicrobial screening of *Dictyopteris polypodioides* methanolic extract was evaluated using the agar disc diffusion method according to NCCLS [30]. A sterile saline solution was inoculated with 18–24 h growth culture of bacteria and fungi strains. The suspension was spread on Petri dishes containing Mueller-Hinton Agar (MHA) for bacteria, Potato Dextrose Agar (PDA) of phytopathologic fungi and Sabouraud dextrose agar (SDA) for yeasts. Then, sterile discs (6 mm in diameter), impregnated with 10µl of algal methanolic extract, were placed on the surface of Petri dishes separately inoculated with different tested strains. As conventional antibiotic (positive controls) gentamicin (15µg/disc) and ciprofloxacin (5µg/disc) were used for bacteria and the antifungal drug fluconazol (40µg/disc) was used as reference for yeasts. Thereafter, the plates were incubated at 37°C for 24 h for bacteria and 28°C for yeasts. The antibacterial and antifungal activities were determined by measuring the diameter of the inhibition zone (mm), formed around the disc.

2.5. Alginate characterization

2.5.1. Extraction

Sodium alginate extraction was performed according to Calumpong et al. [31] modified procedure. Twelve grams of dried algal biomass were soaked in 2% formaldehyde during 24 h at room temperature; thereafter the recovered biomass was washed with distilled water and was put into a solution of 0.2 M HCl for 24 h. After this time, the sample was washed again with distilled water, extracted with 3% sodium carbonate during 24h and filtrated. The filtrate was collected by centrifugation and precipitated by three volumes of ethanol. Sodium alginate recovered was washed by acetone and dried at 50°C.

2.5.2. FTIR Spectroscopy analysis

Sodium alginate samples were dried at 50°C for 3h in an oven before analysis. The infrared spectra were recorded with Thermo Scientific Nicolet Impact 400D FT-IR Spectrometer at room temperature over the wave number range 4000-400 cm^{-1} in an attenuated total reflectance (ATR) mode. A total of 64 scans were averaged for each sample at 4 cm^{-1} resolution and the IR spectra were then plotted and analysed with the OMNIC 7.1 software.

2.5.3 . ^{1}H NMR Spectroscopy analysis

The Na-alginates extracted samples were dissolved in D_2O and stirred prior to NMR spectrum acquisition. ^{1}H NMR analyses were recorded using a spectrometer AV II 400MHz, 9.4T (proton Larmor frequency of 400.33MHz). We used a 5 mm TBI probe. The probe temperature was regulated to 343K. Each spectrum consisted of 16K size of FID covering a sweep width of 4800Hz. We used presaturation during relaxation delay and mixing time. Before Fourier

transformation, the EM apodization functions were applied in one dimension with 0.5 for line broadening. The numbers of scans were 32 transients.

RESULTS AND DISCUSSION

3.1. Total Phenolic compounds

Phenolic compounds are a large and diverse group of molecules, which includes many different families of aromatic secondary metabolites in plants [32]. In brown seaweeds, the only group of tannins present are phlorotannins. These polyphenols are structural classes of polyketides found exclusively in Phaeophycean algae and classified into six groups (Fucol, phlorethols, fucophlorethols, fuhalols, isofuhalols and eckols) based upon variations in their assemblage from the polymerization of phloroglucinol (1,3,5-trihydroxybenzene) units which differ in the number of hydroxyl groups present and in their bond linkages [33,34,35,36]. It has been suggested that phlorotannins become components of brown algal cell walls when physodes fuse with the cell membrane and the phlorotannins are secreted from cells, complexing finally with alginic acid [37,38].

The result of this study shows that *Dictyopteris polypodioides* exhibit higher total phenolic content (27.54 ± 0.59% dry weight) than other brown seaweeds (Table1) such as *Sargassum hystrix* (22±0.91 % dw) [39], *Stypopodium zonale (0.*49±0.04 % dw) [40] and *Sargassum wightii* (2±3.46 % dw) [41]. The variation in total phenolic compound contents (TPC) of all species could be due to the methods used both in sample preparation and extraction [42], since different solvents vary in their extraction efficiency [43,44,32]. According to Ragan and Glombitza, [35] these differences may be due to content of physodes where tissues of brown macroalgae rich in these subcellular structures clearly have higher phlorotannin concentrations than those with less physodes content. On the other hand, variation in TPC of marine macroalgae could be influenced by extrinsic factors: herbivory pressure, irradiance, depth, salinity and nutrients and by intrinsic factors: morphology, age and reproductive stage [45,46,47].

Table 1: Total Phenolic Content (TPC) of *Dictyopteris polypodioides* compared to other brown seaweeds

species	TPC (% dw)	References
Sargassum wightii	2±3.46	[41]
Stypopodium zonale	*0.*49±0.04	[40]
Turbinaria conoides	1.032 ±0.18	[48]
Sargassum hystrix	22±0.91	[39]
Sargassum muticum	20±0.91	[49]
Dictyota cervicornis	5.55±0.23	[50]
Dictyota ciliolata	5.53±0.09	[50]
Lobophora variegata	29.18±0.32	[50]
Dictyopteris polypodioides	27.54±0.59	This study

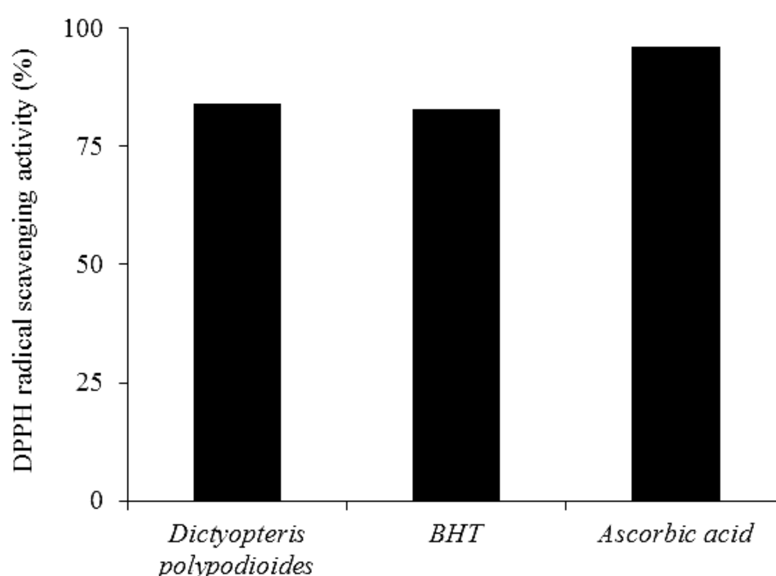

Figure 2: Antioxidant potential of *Dictyopteris polypodioides* extracts determined by DPPH radical-scavenging activity

3.2. DPPH radical scavenging activity

DPPH is commonly used as a free radical to evaluate antioxidant compounds reducing DPPH by donating a hydrogen atom, thereby forming the non-radical DPPH-H [51]. In this study, the methanolic extract of the brown

seaweed *Dictyopteris polypodioides* showed an important radical scavenging activity about 84.13% compared to the positive controls (ascorbic acid: 96.25%; BHT: 83%) (Figure 2). The associated EC_{50} was determined to quantify the radical scavenging, where lowest values of EC_{50} indicate strongest ability of the extract as DPPH scavengers. The seaweed *D. polypodioides* displayed a very low EC_{50} about 0.43 mg/ml compared to some Phaeophycean species as depicted in table 2. The differences observed in EC_{50} values can be explained by the chemical composition of each algal species. According to Stengel and Connan, [52] sources of variability in algal composition can broadly be divided into taxonomic but also ecological and ambient environmental factors. Several reports [53,54,49] suggest that algal phenolic compounds seem to be largely responsible for the antioxidant properties. Recently, Lee et al. [55] and Quéguineur et al. [56] have proved this capacity by *in vitro* evaluation of both algal extracts and isolated compounds using human cell lines. Moreover, Parthiban et al. [57] evaluated the antioxidant capacity of macroalgae extracts and verified that the antioxidant potential was directly proportional to the amount of polyphenols present in the extract.

Table 2: DPPH radical scavenging activity expressed in efficient concentration (EC_{50}) for *Dictyopteris polypodioides* extract and other brown seaweeds

Species	EC_{50} (mg/ml)	Reference
Dictyota crenulata	34.88	[50]
Dictyota ciliolata	12.40	
Turbinaria tricostata	8.85	
Sargassum pteropleuron	7.14	
Sargassum ramifolium	6.64	
Dictyota cervicornis	6.42	
Dictyopteris polypodioides	**0.43**	**This study**

3.3. Antibacterial activity

Methanolic extracts of *Dictyopteris polypodioides* were tested against seven human pathogens (*Staphylococcus aureus, Bacillus cereus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Enterococcus faecalis, Salmonella sp.). D. polypodioides* showed high activity against most of the pathogens tested compared to other seaweeds and to positive control (Table 3). Maximum zone of inhibition was observed against *Bacillus cereus* (36 mm), while minimum activity was noted against *Escherichia coli* (10 mm). The antibacterial activity of seaweeds have been widely discussed in previous studies [58,59,60] while only few ones have been conducted on species belonging to the genus *Dictyopteris*. Mhadhebi et al. [61] for example, reported various activities in extracts of *Dictyopteris membranaceae* against other pathogens (*Staphylococcus epidermidis*, *Staphylococcus aureus and Micrococcus luteus*) and Alghazeer et al. [62] demonstrated pronounced activities in alkaloid extract against *Bacillus subtilis*, *Staphylococcus epidermidis* and *Salmonella typhi*. Like in *D. polypodioides* case, the broad activity of algal extracts against both Gram positive and Gram negative bacteria may be indicative of the presence of wide spectrum antibiotic compounds or simply the content of pharmacological active constituents like alkaloids, saponins, glycosides, tannins [59]. Furthermore, the phlorotannins (eckol, dieckol, 8,8'-bieckol, phlorofucofuroeckol A, fucofuroeckol A, dioxinodehydroeckol and 7-phloroeckol) specifically isolated from brown seaweeds have been proved as antibacterials [63,64,65,66]. Although the mechanism by which phlorotannins exert antibacterial activity has not been defined. It is believed that, as verified for terrestrial tannins, these compounds can act by forming complexes with bacterial proteins and carbohydrates and inhibit extracellular microbial enzymes [67,68].

Table 3: Antibacterial activity of *Dictyopteris polypodioides* compared to other seaweeds and antibiotics

	Inhibition zone diameter (mm) *				
	Seaweeds			Antibiotics	
Microorganisms	***Dictyopteris polypodioides***	*Dictyosphaeria cavernosa*	*Codium decorticatum*	**Gentamicin (15 µg/disc)**	**Ciprofloxacin (5µg/disc)**
Gram negative					
Escherichia coli	**10**	13	24	**25**	**32**
Pseudomonas aeruginosa	**15**	NT	NI	**20**	**31**
Klebsiella pneumoniae	**14**	16	26	**NT**	**7**
Salmonella sp.	***11***	NT	NT	**20**	**10**
Gram positive					
Enterococcus faecalis	**12**	NT	NT	**NT**	**NT**
Staphylococcus aureus	**28**	18	NI	**31**	**27**
Bacillus cereus	**36**	NT	NT	**40**	**27**
References	**This study**	[59]	[60]	**This study**	**This study**

** Inhibition zone including disc diameter (6 mm).*
NI: no inhibition, NT: not tested.

3.4. Antifungal activity

Seaweeds are directly exposed in the various oceanic environment conditions and are supposed to be susceptible to ambient microorganisms. However, they have incredible survival ability because they possess an inherently

available chemical defense mechanism. Accordingly, many bioactive compounds (e.g., terpenes, phlorotannins,..) could be present [69]. Currently the search for new antifungal agents is a growing need, owing to the increment of fungal infections, but also because of the increase in resistance to antifungal agents [70]. Consequently, large attention has been given to natural products with antifungal properties. In the present work the methanolic extract of *Dictyopteris polypodioides* has been tested against four yeast strains of *Candida* and five phytopathogenic fungi (Table 4). The obtained results showed that *D. polypodioides* exhibited strong antifungal activities with diameters of growth inhibition ranging between 7 and 20 mm against phytopathogenic fungi and between 12 and 32 mm against the tested yeasts. The antifungal activity of seaweeds extracts and isolated compounds has not been extensively studied, mainly because in the past few years more attention has been paid to pathogenic bacteria, which is, by far, more explored [71]. Nevertheless, the antifungal activity of seaweeds has been demonstrated by some researchers where the results showed that fungal inhibition depends on seaweeds species and solvent used. For example, Guedes et al. [72] was found that methanolic, ethanolic and dichloromethanolic extracts of seaweeds were most active against dermatophytes and *Candida sp.* compared to other solvents. According to Cowan et al. [73], the brown seaweeds contain high amount of flavanoid and phenolic compounds could be the reason for antifungal activity. Furthermore, a meroditerpenoid metabolite isolated and characterized from the brown alga *Cystoseira tamariscifolia* as Methoxybifurcarenone posses antifungal activity against three tomato pathogenic fungi, *Botrytis cinerea*, *Fusarium oxysperium sp. mycopersici* and *Verticillium alboatrum* [74]. Long before, Fenical *et al.* [75] reported that the two sesquiterpenes zonarol and isozonarol from *Dictyopteris zonarioides* strongly inhibited the growth of ten species of pathogenic fungi causing diseases in plants.

Table 4: Antifungal activity of methanolic extract of *Dictyopteris polypodioides*

	Inhibition zone diameter (mm) *	
Microorganisms	*Dictyopteris polypodioides*	Fluconazol (40 µg/disc)
Yeasts		
Candida albicans	12	25
Candida glabrata	32	26
Candida krusei	18	24
Candida parapsilosis	26	27
Phytopathogenic fungi		
Verticillium dahliae	10	NT
Fusarium oxysporum	9	NT
Fusarium graminearum	8	NT
Botrytis cinerea	10	NT
Geotrichum sp.	10	NT

** Inhibition zone including disc diameter (6 mm).*
NT: not tested.

3.5. Alginate characterization

3.5.1. Alginate extraction yield

Sodium alginate yield for *Dictyopteris polypodioides* was about 21.92 % dw which seem to be intersting compared to the literature data since, for feasible commercial exploitation, the seaweed needs to contain at least 20% alginate based on dry weight [76]. This obtained result is similar to some well-known worldwide alginophytes (*Macrocystis pyrifera* 18-21% dw, *Laminaria japonica* 20-26 % dw; [77]) but remains lower compared to the exceptional contents in *Durvillaea antarctica* (53 % dw; [77]) and *Ecklonia cava* (35-38 % dw; [78]. Besides the inter-species variations, alginate levels may vary, however, according to geographic area, season or environmental conditions [79].

3.5.2. FTIR analysis

Sodium alginate from *Dictyopteris polypodioides* shows a high similarity to commercial alginate (Figure 3) where abroad band at 1600 cm^{-1} was assigned to carboxylate O–C–O asymmetric stretching confirming the high uronic acid content of both biopolymers [80]. According to Mathlouti and Koening,[81] and to Silverstein et al. [82], the absorption at 1415 cm^{-1} was assigned to C–OH deformation vibration with contribution of O–C–O symmetric stretching vibration of carboxylate group. The bands measured at 1030 cm^{-1} may be attributed to C–O and (C-C) stretching vibrations of pyranose rings, [83]. The anomeric region (950–750 cm-1) is the most discussed in carbohydrates. Spectrum of *D. polypodioides alginate* shows a peak at 946 cm^{-1}, which was assigned to the C–O stretching vibration of uronic acid residues [84]. The signal at 905 and 850 cm^{-1} were assigned to the α-L-gulopyranuronic asymmetric ring vibrationand and to the C1–H deformation vibration of β-mannuronic acid residues respectively [81,85]. Finally, the signal at 782 cm^{-1} seems to be characteristic of guluronic acid residues [85].

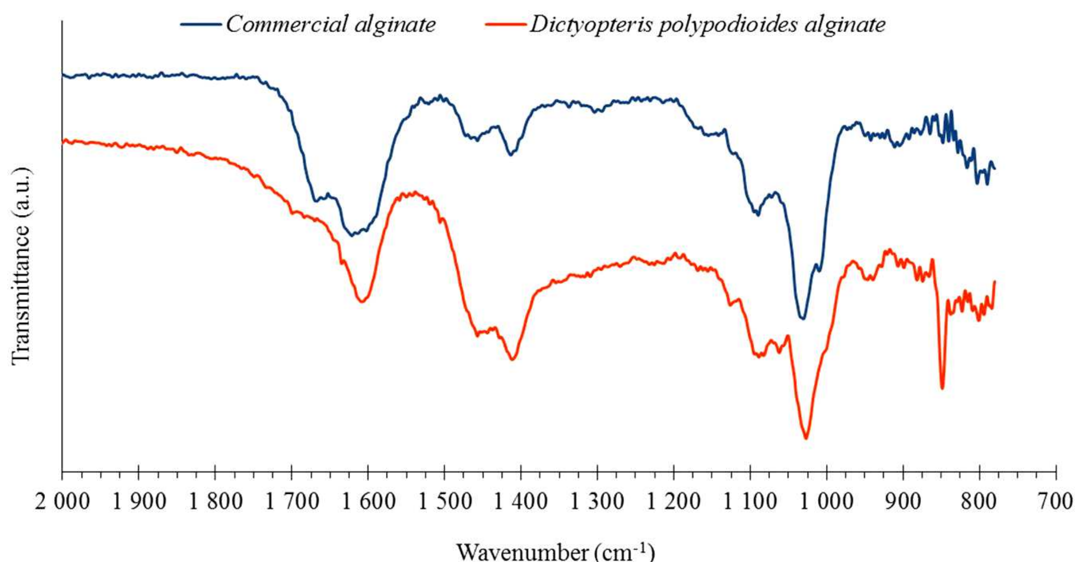

Figure 3: FTIR spectra of *Dictyopteris polypodioides* and commercial sodium alginates

3.5.3. ^{1}H NMR characterization

^{1}H NMR spectroscopy is a reliable method for determination of the composition and block structure of alginate [86,87]. Figure 4 shows three characteristics signals assigned to the guluronic acid anomeric proton (G-1) at 5.06 ppm, mannuronic acid anomeric protons (M-1M and M-1G) and the C-5 of alternating blocks (G-5M) at 4.7 ppm and guluronic acid H-5 (G-5) overlapped at 4.4 ppm. The ratio of mannuronic acid to guluronic acid, M/G, and the molar fractions of the monads of guluronic acid and mannuronic acid (F_G, F_M) as well as the diad sequences (F_{GG}, F_{MM}, F_{MG}, F_{GM}) were determined from the area of ^{1}H NMR signals (Figure. 4) and using the equations proposed by Grasdalen [88] and Grasdalen et al. [89] as follow:

F_G = **[** G-1 Signal area **]** / **[** (M-1+G-5M) signal area + GG-5G signal area **]**
$F_M = 1 - F_G$
F_{GG} = **[** GG-5G signal area **]** / **[** (M-1+G-5M) signal area + GG-5G signal area **]**
$F_{MG} = F_{GM} = F_G - F_{GG}$
$F_{MM} = F_M - F_{MG}$
$M/G = F_M / F_G = (1 - F_G) / F_G$
$\eta = F_{MG} / (F_M \times F_G)$

The comparison of the M/G ratio for *Dictyopteris polypodioides* with M/G from other brown seaweeds species are given in Table 5. The obtained M/G ratio for *Dictyopteris polypodioides* was about 0.7 indicating high guluronic acid content. This result is similar to those reported for several brown species such as *Laminaria hyperborea* (0.82), *Saccharina longicruris* (0.69), *Sargassum thunbergii* (0.53), *Sargassum latifolium (0.82) and Sargassum asperifolium* (0.69) (Table 5). According to Penman et al. [90], the ability to form gels by alginates is markedly influenced by the uronic acid composition, where brittle gels are obtained from alginates with a low M/G ratio, while elastic gels are formed from alginates with a high M/G ratio. The variation of this ratio depends mainly on algal species, geographical location and extraction protocols [91]. It is not only the M/G ratio which has an influence on alginate gelling properties, but also the percentages of homopolymeric block structures (F_{MM}, F_{GG}) and alternating blocks ($F_{MG,}$ F_{GM}) [92]. *Dictyopteris polypodioides* showed high values for the alternating blocks MG and GM ($F_{MG} = 0.41$, $F_{GM} = 0.41$) and lower values of the homopolymorphic regions ($F_{GG} = 0.18$, $F_{MM} = 0.01$). This results has been confirmed by the use of the parameter η = FMG / (FM × FG), which characterize and test the sequence distributions. According to Grasdalen et al. [89] biopolymers with $1 < \eta < 2$, have a sequence distribution of the alternate block type (MG, GM). The calculated η (1.67) for *Dictyopteris polypodioides* means that the extracted alginate is an alternated polymer which corroborates alginate distributions in *Saccharina longicruris (*η = 1.41, [93]), *Sargassum thunbergii (*η = 1.52, [91]*), Sargassum vulgare (*η = 1.75, [92]).

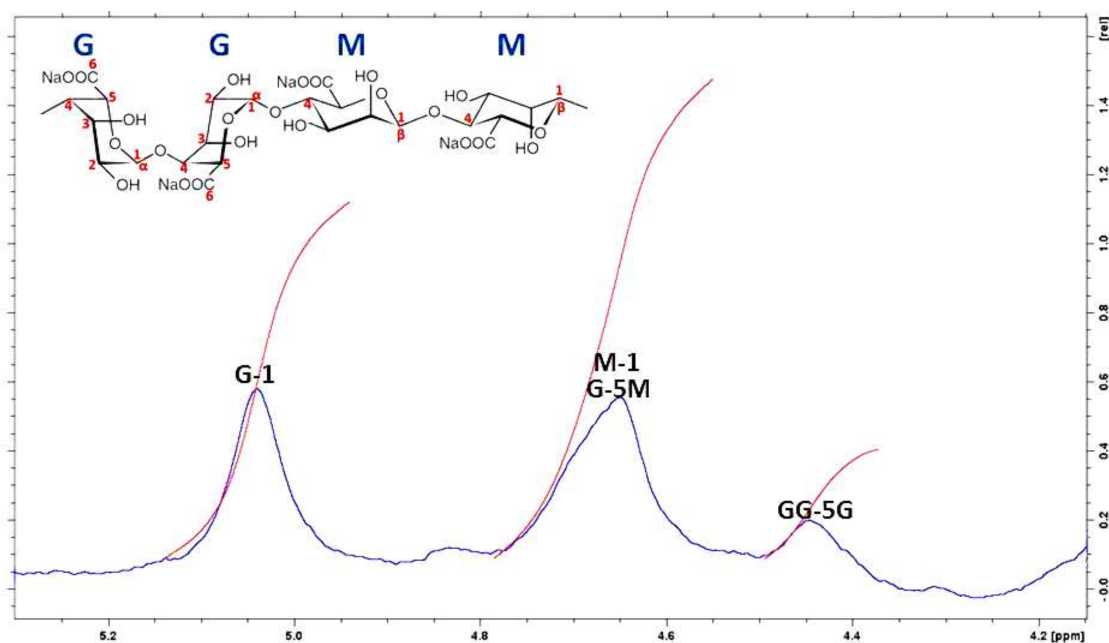

Figure 4: 400 MHz - ^{1}H NMR spectrum of sodium alginate (in D_2O at 343K) extracted from *Dictyopteris polypodioides* and signal assignments for protons on carbon-1 and carbon-5 of mannuronic acid (M) and guluronic acid (G) residues

Table 5: Alginate composition of *Dictyopteris polypodioides* and other brown seaweeds

Species	M/G	F_M	F_G	F_{MM}	F_{GG}	F_{GM}	F_{MG}	η	Reference
Laminaria japonica	1.86	0.65	0.35	0.48	0.18	0.17	0.17	0.75	[94]
Laminaria digitata	1.44	0.59	0.41	0.43	0.25	0.16	0.16	0.66	[94]
Laminaria hyperborea	0.82	0.45	0.55	0.28	0.38	0.17	0.17	0.69	[95]
Durvillaea willana	2.57	0.52	0.48	0.52	0.08	0.20	0.20	0.8	[87]
Macrocystis pyrifera	1.70	0.63	0.37	0.40	0.14	0.23	0.23	0.99	[87]
Saccharina longicruris	0.69	0.41	0.59	0.07	0.25	0.34	0.34	1.41	[92]
Sargassum thunbergii	0.53	0.34	0.66	0.17	0.48	0.34	0.34	1.52	[91]
Sargassum vulgare	1.27	0.56	0.44	0.02	0.55	0.43	0.43	1.75	[92]
Sargassum latifolium	0.82	0.45	0.55	0.41	0.51	0.04	0.04	0.16	[86]
Sargassum asperifolium	0.69	0.41	0.59	0.30	0.48	0.11	0.11	0.45	[86]
Sargassum muticum	0.31	0.24	0.76	0.07	0.59	0.17	0.17	0.93	[91]
Sargassum turbinarioides	0.94	0.48	0.52	0.36	0.39	0.12	0.12	0.48	[83]
Dictyopteris polypodioides	**0.70**	**0.41**	**0.59**	**0.01**	**0.18**	**0.41**	**0.41**	**1.67**	**This study**

CONCLUSION

Seaweeds or marine macroalgae have become in recent years a key focus area for the discovery of new compounds and natural products for use in the food and pharmaceutical industries. In this work, methanolic extract of the brown seaweed *Dictyopteris polypodioides* harvested from the Atlantic coast of Morocco, was screened for its *in vitro* bioactivity, antioxidant potential, total phenolic content and alginate properties. Obtained results showed that high antibacterial inhibitory activities were recorded against Gram positive (12-36 mm) and Gram negative pathogenic bacteria (10-15 mm). Methanolic extract of *Dictyopteris polypodioides* also exhibits high antifungal activities against tested phytopathogenic fungi (8-10 mm) and yeast (12-32 mm). *Dictyopteris polypodioides* showed important radical scavenging activity (84.13%) and total phenollic content (27.54±0.59 % dw). Furthermore, alginate yield of this brown algae was important (21.92 % dw), characterized by more guluronic acid than mannuronic acid (M/G = 0.7) and by a dominance of the heteropolymorphic diads MG and GM (82%, η = 1.67). The brown seaweed *Dictyopteris polypodioides* could be a promising source of interesting bioactive molecules and alginate for commercial uses. However, further studies on fractionation and separation are required to identify the principle active molecules.

REFERENCES

[1] S.K. Kim, Handbook of Marine Macroalgae: Biotechnology and Applied Phycology. JohnWiley and Sons, UK, **2012**, 567.
[2] V. Besada, J.M Andrade, F Schultze, *J.Mar .Syst*., **2009**, 75, 305–313.

[3] J.O Muse, J.D Stripeikis, F.M Fernandez, L. d'Huicque, M.Tudino, C.N Carducci, E*nv.Poll.*, **1999**,104(2), 315–322.
[4] G.M.A. Filho, L.R. Andrade, C.S. Karez, M. Farina, W.C Pfeiffer, *Mar. Env. Res*., **1999**,48(3), 213–224.
[5] C.S. Karez, V.F. Magalhaes, W.C. Pfeiffer, G.M.A. Filho, *Env. Pol.*, **1994**, 83(3),351–356.
[6] V. Venugopal, Marine Polysaccharides Food Applications, CRC Press, Taylor and Francis Group. 2011, 352.
[7] M. Tori, K. Nakashima, M. Seike, Y. Asakawa, A.D. Wrigh, G.M König, O. Sticher. *Tetrahedron. Lett*., **1994**, 35, 3105–3106.
[8] G. Guella, A. Oztunc, I. Mancini, F. Pietra, *Tetrahedron Lett*., **1997**, 38,8261–8264.
[9] S.R Gedara, O.B Abdel-Halim, S.H El-Sharkawy, O.M Salama, T.W Shier, A.F Halim, *Z. Naturforsch. C*., **2003**, 58, 17–22.
[10] C.E Goez, A.D. Wright, G.M. Koenig, O.Stiche, *Phytochem. Anal*., **1994**, 5, 68–73.
[11] C. Areche, A. San-Martin, J. Rovirosa, J. Soto-Delgado, R. Contreras, *Phytochemistry.*, **2009**, 70, 1315–1320.
[12] M. Kladi, C. Vagias, M. Stavri, M. Rahman, S. Gibbons, V. Roussis, *Phytochem. Lett*, **2008**, 1,31–36.
[13] X. Xu, F. Song, S.Wang, S. Li, F. Xiao, J. Zhao, Y. Yang, S. Shang, L.Yang, J. Shi, *J. Nat. Prod*., **2004**, 67,1661–1666.
[14] B.G. Fleury, M.V.G. Pereira, J.R.P. Da Silva, M. Kaisin, V.L. Teixeira, A. Kelecom, *Phytochemistry.*, **1994**, 37,1447–1449.
[15] Z. Kamenarska, M.J. Gasic, M. Zlatovic, A. Rasovicd, D. Sladicb, Z. Kljajicd, K. Stefanova, K. Seizovaa, H. Najdenskic, A. Kujumgievc, I. Tsvetkovac, S. Popova, *Bot. Mar.*, **2002**, 45, 339–345.
[16] N. Xu, X. Fan, X.Yan, C. K.Tseng, *J. Appl. Phycol*., **2004**, 16,451–456.
[17] A.G Del Val, G. Platas, A. Basilio, A. Cabello, J. Gorrochategui, J. Suay, F.Vicente, E. Portillo, M.J. Del Río, G. García Reina, F.Peláez, *Int. Microbiol.*, **2001**, 4,35–40.
[18] C.S. Vairappan, S.P Anangdan, K.L. Tan, S. Matsunaga. *J. Appl. Phycol*., **2010**, 22, 305–311.
[19] G.M. Nylund, F. Persson, M. Lindegarth, G. Cervin, M. Hermansson, H. Pavia, *Fems. Microbiol. Ecol*., **2010**, 71(1), 84–93.
[20] K. Li, X.M. Li, N.Y. Ji, B.G. Wang, *Bioorg. Med. Chem.*, **2007**, 15, 6627–6631.
[21] R. Chatter, M. Kladi, S. Tarhouni, R. Maatoug, C. Vagias, V. Roussis, *Phytochem. Lett.*, **2009**, 2, 25–28.
[22] A.K. Sen, A.K. Das, N. Banerji, A.K. Siddhanta, K.H. Mody, B. K Ramavat, V.D. Chauhan, J.R. Vedasiromoni, D.K. Ganguly. *Int. J. Biol. Macromol*., **1994**, 16, 279–280.
[23] J.W. Blunt, B.R. Copp, M.H.G. Munro, P.T. Northcote, M.R. Prinsep, *Nat. Prod. Rep*., **2006**, 23, 26–78.
[24] H.S. Pereira, L.R. Leao-Ferreira, N. Moussatche, V.L. Teixeira, D.N Cavalcanti, L.J. Costa, R. Diaz, I.C.P.P. Frugulhetti, *Antiviral. Res*., **2004**, 64, 69–76.
[25] N. Karaki, C. Sebaaly, N. Chahine, T. Faour, A. Zinchenko, S. Rachid, H. Kanaan, *J App Pharm Sci.*, **2013**, 3 (02), 043–051.
[26] K.D. Magalhaes, L.S. Costa, G.P Fidelis, R.M. Oliveira, L. T. D. Barreto Nobre, N. Dantas-Santos, R.F.G. Camara, I. R. L. Albuquerque, S.L. Cordeiro, D.A. Sabry, M.S.S. Pereira Costa, L.G. Alves, H.A.O. Rocha, *Int. J. Mol. Sci*., **2011**, 12, 3352–3365.
[27] A.D. Kim, K.A. Kang, M.J. Piao, K.C. Kim, J. Zheng, C.W. Yao, J.W. Cha, C.L Hyun, S.J. Boo, N.H. Lee, Y.S. Na, J.W. Hyun, *Biotechnol. Bioprocess. Eng*., **2014**, 19, 419–425.
[28] N. Heffernan, T.J. Smyth, A. Soler Villa, R.J. Fitzgerald, N.P. Brunton, *J. Appl. Phycol*., **2014**, 27(1), 519–530.
[29] M.S. Blois, *Nature.*, **1958**, 181, 1199-1200.
[30] NCCLS, National committee for clinical laboratory standards, Performance standards for antimicrobial disk susceptibility test, 6th Ed Approved Standard M2-A6, **1997.**
[31] P.H. Calumpong, P.A. Maypa, M. Magbanua, *Hydrobiologia*., **1999**, 398, 211–215.
[32] P.G. Waterman, S. Mole, Analysis of phenolic plant metabolites. Ed Blackwell Scientific Publications.Wiley-Blackwell, Oxford, UK, **1994,** 248.
[33] J.A. Geiselman, O.J. McConnell, *J. Chem. Ecol*., **1981**, 7, 1115–1133.
[34] J. Grosse Damhues, K.W Glombitzka, *Phytochemistry*., **1984**, 23, 2639–2642.
[35] M.A. Ragan, K.W. Glombitza, In F.E. Round, D.J. Chapman (Eds.), Phlorotannins, brown algal polyphenols (Progress in Phycological Research, 1986) 241**.**
[36] N. M. Targett, T.M Arnold, *J. Phycol*., **1998**, 34,195–205.
[37] T.M. Arnold, N.M. Targett, *Oikos.,* **2003**, 100**,** 406-408.
[38] M. E. A. Schoenwaelder, M.N. Clayton, *J Phycol.,* **1998**, 34**,** 969-980.
[39] S.A. Budhiyanti, S. Raharjo, D.W. Marseno, I.Y.B. Lelana, *Am. J. Agric. Biol. Sci.*, **2012**, 7 (3), 337-346.
[40] G. Ank. W. Da Costa Paradas, G.M. AmadoFilho, B.A. Perez Da Gama, R.C. Pereira, *Pan. Am. J. Aquat. Sci.*, **2014**, 9(1), 1–7.
[41] G. Kokilam, S. Vasuki, N. Sajitha, *J. App. Pharm. Sci.,* **2013**, 3 (11), 099–104.
[42] K.L. Lann, C. Jegou, S.P Valerie, *Phycol. Res.,* **2008**, 56, 238–245.
[43] R. Koivikko, *J. Chem. Ecol*., **2008**, 34**,** 57–64.

[44] R. Koivikko, J. Loponen, T. Honkanen, V.Jormalainen, *J. Chem. Ecol*., **2005**, 31**,** 195–212.
[45] Y.L. Chew, Y.Y. Lim, M. Omar, K.S. Khoo, *Food. Chem*., **2008**, 41, 1067–1072.
[46] K Ganesan, C.S Kumar, P.V.S. Rao, *Innov. Food. Sci. Emerg. Technol*., **2011**, 12, 73–78.
[47] K.L. Lann, C. Ferret, E. Vanmee, C. Spagnol, M. Lhuillery, C. Payri, S.P. Valerie, *Phycol. Res*., **2012**, 60,37–50.
[48] C. Parthiban, C. Saranya, S.T. Somasundaram, P. Anantharaman, *Int. J. Phyto. Pharm*., **2014**, 5(1), 36–41.
[49] A. Tanniou, S.L Esteban, L. Vandanjon, E. Ibanez , J.A. Mendiola, S. Cerantola , N. Kervarec, S. La Barre, L. Marchal, S.P. Valerie, *Talanta.*, **2013**, 104, 44–52.
[50] M. Zubia, D. Robledo, Y. Freile-Pelegrin, *J. Appl. Phycol.,* **2007**, 19, 449–458.
[51] M.L. Cho, H.S. Lee, I.J. Kang, M.H. Won, S.G. You, *Food. Chem*., **2011**, 127, 999–1006.
[52] D.B. Stengel, S. Connan, Natural products from marine algae: methods and protocols, Humana Press , Springer New York, **2015**, 439.
[53] S. Connan, F. Delisle, E. Deslandes, E. Ar Gall, *Bot. Mar*., **2006**, 49, 34–46.
[54] S.J. Kim, S. Woo, H. Yun, S. Yum, E. Choi, J.R. Do, J.H. Jo, D. Kim, S. Lee, T.K. Lee, *Food. Sci. Biotechnol*., **2005**, 14, 798–802.
[55] M.S. Lee, T. Shin, T. Utsuki, J.S. Choi, D.S. Byun, H.R. Kim, *J Agric. Food. Chem*., **2012**, 60(21), 5340–5349.
[56] B. Quéguineur, L. Goya, S. Ramos, M.A. Martín, R. Mateos, M.D. Guiry, L. Bravo, *J. Appl. Phycol*., **2013**, 25(1), 1–11.
[57] P. Karthick, R. Mohanraju, M.K. Narayana, C.H Ramesh, *J. Algal. Biomass. Utln*., **2015**, 6 (1),33–36.
[58] W. Boonchum, Y. Peerapornpisal, D. Kanjanapothi, J. Pekkoh, D. Amornlerdpison, C. Pumas, P. Sangpaiboon, P. Vacharapiyasophon, *Int. J. Agric. Biol*., **2011**, 13, 100–104.
[59] C. Parthiban, C. Saranya, K. Girija, A. Hemalatha, M. Suresh, P. Anantharaman, *Int. J Curr. Microbiol. App. Sci*., **2013**, 2(9), 64–73.
[60] J.A.J. Sunilson, R. Suraj, K. Anandarajaopal, G. Rejitha, M. Vignesh, P. Promwichit, *Int. J. Biol. Chem*., **2009**, 3, 84–89.
[61] L. Mhadhebi, K. Chaieb, A. Bouraoui, *Int. J. Pharm. Pharm. Sci*., **2012**, 4(1), 534–537.
[62] R. Alghazeer, W. Fauzi, A. Entesar, F. Gammoudi, M. Naili, *Afr. J. Biotechnol.*, **2013**, 12(51), 7086–7091.
[63] K. Nagayama, Y. Iwamura, T. Shibata, I. Hirayama, T. Nakamura *J. Antimicrob. Chemoth*., **2002**, 50(6), 889–893.
[64] S.H. Eom, Y.M. Kim, S.K. Kim, *Food. Chem. Toxicol*., **2012**, 50(9), 3251–3255.
[65] J.G. Choi, O.H. Kang, O.O. Brice, Y.S. Lee, H.S. Chae, Y.C. Oh, D.H. Sohn, H. Park, H.G. Choi, S.G. Kim, D.W. Shin, D.Y. Kwon, *Foodborne. Pathog. Dis*., **2010**, 7(4), 435–441.
[66] M. Lee, K. Lee, S. Oh, B. Lee, H. Chee, *J. Korean. Soc. Appl. Biol. Chem*., **2010**, 53(4), 504–507.
[67] J. Schultz, M. Hunter, H. Appel. In: R. W. Hemingway, P.E. Laks (Eds.), Antimicrobial Activity of Polyphenols Mediates Plant-Herbivore Interactions (Plenum Press, New York, **1992**) 621-637.
[68] Y. Wang, Z. Xu, S. Bach, T. McAllister, *Asian. Austral. J. Anim*., **2009**, 22(2), 238–245.
[69] Y. Peng, J. Hu, B. Yang, X.P. Lin, X.F. Zhou, X.W. Yang, Y.H. Liu, In Elsevier Science (Ed.), Seaweed Sustainability: Food and Non-Food Applications (Academic Press, **2015**) 472.
[70] M.D. Richardson, D.W Warnock, Fungal infection: diagnosis and management (Wiley-Blackwell, USA, **2012**) 476.
[71] A. Mayer, A. Rodríguez, O. Taglialatela-Scafati, N. Fusetani, *Mar. Drugs.*, **2013**, 11(7), 2510–2573.
[72] E.M.C. Guedes, M.A. dos Santos Araújo, A.K.P. Souza, L.I.S. Souza, L.D. Barros, F.C. Albuquerque Maranhão, Goulart A.E. Sant'Ana, *Mycopathologia.,* **2012**,174(3), 223–232.
[73] M.M. Cowan, *Clin. Microbiol. Rev*., **1999**, 12, 564–582.
[74] A. Bennanmara, A. Abourriche, M. Berrada, M. Charrouf, N. Chaib, M. Boundouma, F.X. Garjeall, *Phytochemistry*., **1999**, 52, 37–40.
[75] W. Fenical, J.J. Sim, D. Squatrito, R.M. Wing, P. Radlick, *J. Org. Chem*., **1973**, 38, 2383–2385.
[76] M. Rinaudo, In: J.P Kalmerling (Ed.), Seaweed polysaccharides, Comprehensive glycoscience: From chemistry to systems biology (Elsevier**, 2007)** 742.
[77] R. Pérez, Ces algues qui nous entourent. Conception actuelle, rôle dans la biosphère, utilisations, culture (Ifremer, Fr, **1997**) 272.
[78] I.J. Miller, *Phytochemistry*., 1996, 41(5), 1315–1317.
[79] L.L. Mafra, S.R. Cunha, *J. Coast. Res*., **2006**, 39, 1847–1852.
[80] F. Bi, S.J. Mahmood, M. Arman, N. Taj, S. Iqbal, *Phys. Chem. Liq*., **2007**, 45 (4), 453- 461.
[81] M. Mathlouthi, J.L. Koening, *Adv. Carbohydr. Chem. Biochem*., **1986**, 44, 7–89.
[82] R.M. Silverstein, G. Clayton Bassier, TC. Morril, Spectrometric identification of organic compounds (Wiley, New York, **1991**) 419.
[83] T.A. Fenoradosoa, G. Ali, C. Delattre, C. Laroche, E .Petit, A. Wadouachi, P. Michaud. *Appl. Phycology*., **2010**, 22, 131–137.
[84] N.P. Chandía, B. Matsuhiro, A.E. Vásquez, *Carbohydr. Polym*., **2001**, 46, 81–87.

[85] S.K. Papageorgiou, E. P. Kouvelos, E.P. Favvas, A.A. Sapalidis, G.E. Romanos, F.K. Katsaros, *Carbohydr. Res*., **2010**, 345, 469–473.
[86] B. Larsen, M.S.A. Salem Dalia, M.A.E. Sallam, M.M. Mishrikey, A.I. Beltagy. Carbohydr. Res., **2003**, 338, 2325–2336.
[87] R. Panikkar, D.J. Brasch, *Carbohydr. Res*., **1996**, 293, 119–132.
[88] H. Grasdalen, *Carbohydr. Res*., **1983**, 118, 255–260
[89] H. Grasdalen, B. Larsen, O. Smidsrod, *Carbohydr. Res*., **1979**, 68, 23–31.
[90] A. Penman, G.R. Sanderson. *Carbohydr. Res*., **1972**, 25, 273–282.
[91] T.A. Davis, F. Llanes, B. Volesky, A. Mucci, Environ. Sci. Technol., **2003**, 37, 261–267.
[92] M.R. Torres, A.P.A. Sousa, E.A.T. Silva Filho, D.F. Melo, J.P.A. Feitosa, R.C.M. de Paulab, M.G.S. Lima, *Carbohydr. Res*., **2007**, 342(14), 2067–2074.
[93] L.E. Rioux, S.L. Turgeon, M. Beaulieu, *Carbohydr. Polym*., **2007**, 69, 530–537
[94] O. Smidsrød, K.I. Draget, *Carbohydr. Eur*., **1996**, 14, 6–13.
[95] A. Martinsen, G. SkjBk-Braek, O. Smidsrsd, *Biotechnol. Bioeng*., **1989**, 33(1), 79–89.